APRIL 1997

# AUSTRALIAN QUARANTINE REQUIREMENTS FOR THE IMPORTATION OF BOVINE EMBRYOS FROM THE UNITED STATES OF AMERICA

#### 1. GENERAL

- 1.1 Each consignment of bovine embryos must be accompanied by a copy of a valid *Permit to Import semen/embryos into Australia* obtained, prior to the export of the embryos, from the Australian Quarantine and Inspection Service (AQIS) office in the State of Australia to which the importation is to be made.
- 1.2 Each consignment must be accompanied by an Animal Health Certificate which conforms to the template shown at Attachment 1. It must be in English and signed by both the Embryo Collection Team Veterinarian and an Official Veterinarian. The Team Veterinarian must be registered with the United States Department of Agriculture (USDA) as approved to collect embryos for export. An Official Veterinarian is a civil service veterinarian or a specially appointed veterinarian as authorized by the USDA to certify on behalf of the US Federal Government.

The embryos must be fertilized  $in\ vivo$ , collected and processed by a USDA approved embryo collection team and meet the requirements specified in Section 2 of this document. This must be certified under V. Sanitary information in the Animal Health Certificate.

The Certificate must be stamped on each page with an Official stamp.

\*\*\*NOTE: The certificate to be used is included in this document.

- 1.3 The requirements in Section 2 of this document are the minimum requirements for importation into Australia. Different zones of Australia have different animal health status and State or Territory veterinary authorities may require further testing or certification for certain diseases before movement into a specific zone.
- 1.4 Relevant World Organization for Animal Health (OIE) International Animal Health Code (OIE Code) Articles defining disease freedom and OIE Code Appendices relating to collection and processing of bovine embryos are at Attachment 2.
- 1.5 All samples collected for diagnostic tests, all collections of embryos and all servicing of storage containers prior to export must be performed under the direct supervision of either the Team Veterinarian or the Official Veterinarian. The program may be subject to direct audit by AQIS at any time during the collection period.
- 1.6 The final identification and placement of the embryos into new, unused liquid nitrogen in a new or properly disinfected container prior to export of the embryos to Australia must be performed under the supervision of an Official Veterinarian.
- 1.7 The embryos must be shipped to the Australian importer care of AQIS.
- 1.8 Conditions of importation may be varied or reviewed at any time at the discretion of AQIS.

#### 2. CERTIFICATION

The Animal Health Certificate must attest under V. Sanitary Information, that:

- 2.1. The United States of America is recognized by the OIE as a foot-and-mouth disease (FMD) free country where vaccination is not practiced, and meets the OIE Code Article definitions for country freedom from:
  - rinderpest (Article 2.1.4.2)
  - contagious bovine pleuropneumonia (Article 2.1.6.2)
  - lumpy skin disease (Article 2.1.7.2)
  - Rift Valley fever (Article 2.1.8.2), and
  - bovine spongiform encephalopathy (BSE) (Article 3.2.13.2).
- 2.2. None of the male of female donors has resided in the United Kingdom.
- - was born after the prohibition in that country, on feeding to ruminants animal feeds containing tissues originating from ruminants; has not been resident, since weaning, on a property or premises on which meat meal was fed to ruminants since the respective donor was born;
  - has not originated from, or been part of, a herd in which there has been a confirmed case of BSE;
  - is not the offspring of a BSE suspect or affected dam.
- 2.4. Each female donor had been continually resident and free from any quarantine restrictions in the United States of America (USA) or Canada for at least 90 days immediately prior to the first collection of embryos.
- 2.5. Each female donor was inspected by the Team Veterinarian or an Official Veterinarian on each day that embryos were collected for this consignment and was found to be free from signs of infectious or contagious disease.
- 2.6. Bovine pestivirus (BVD) testing Each female donor gave a negative result to one of the following tests for bovine pestivirus using a blood specimen taken between 30 and 14 days immediately prior to embryo collection: EITHER
  - an antigen capture enzyme-linked immunosorbent assay (ELISA) on peripheral blood leucocytes,

OR

- a monoclonal immunoperoxidase (IP) or other virus isolation test on blood or serum.
- 2.7. Bovine tuberculosis is compulsorily notifiable in the USA.

During the 180 days immediately prior to and during embryo collection, the herds in which each female donor resided were officially free from bovine tuberculosis as defined by OIE Code Article 3.2.3.1., and/or Accredited (tuberculosis free) by the USDA.

- 2.8. Vesicular stomatitis was not reported within 80 kilometers of the premises where the male or female donors resided during the period from two months prior to the first collection for this consignment until one month after the final collection for this consignment.
- 2.9. The semen used to produce the embryos in this consignment was free from quarantine restrictions and was: EITHER:
  - collected at a semen collection center which complies with "CSS (Certified Semen Services) Minimum Requirements for Disease Control of Semen Produced for AI."

- complied with the minimum health standards for semen imported into the USA.
- 2.10. All the embryos in this consignment were fertilized in vivo, collected, processed, identified, stored and transported in accordance with Code Appendix 4.2.3.1., by an embryo collection team which met the Code conditions and was approved, at the time of collection, by the USDA for the collection of embryos for export. The embryos were treated with trypsin during the washing process as described in Section IV Chapter 2 of the IETS Manual.
- 2.11. The embryos in this consignment were EITHER
  - not subjected to micromanipulation involving breaching of the zona pellucida and all had intact zona pellucida at the time of storage  $_{\mbox{\scriptsize OR}}$
  - or met the conditions laid out in Code Appendix 4.2.3.5. for micromanipulated embryos.
- 2.12. The embryos in this consignment have been stored:
  - only with other embryos or semen collected for export to Australia;
  - in sealed containers;
  - since the end of the collection period until export in an approved secure place.
- 2.13. The embryos were identified and placed into new, unused liquid nitrogen in a new or properly disinfected container for export under the supervision of an Official Veterinarian. The container was sealed and the number or mark on the seal recorded on the certificate prior to export.

#### 3. IMPORTERS/AGENTS RESPONSIBILITIES

- 3.1 It is the prerogative of the importer to arrange for any other health certification or testing of donors (eg for inherited diseases or genetic defects or for movement of animals or genetic material into certain zones in Australia).
- 3.2 The importer must nominate a person who will be accessible to AQIS and who will accept responsibility for ensuring that all import requirements are met and for Customs clearance and Quarantine inspection and clearance in Australia.

#### 4. POST ARRIVAL

- 4.1 The consignment will be held by AQIS until a Quarantine Officer has checked the certification and conducted an audit of the contents of the shipping container.
- 4.2 In the event of a consignment arriving in Australia without the correct certification, with the seals on the transport containers broken or in any other way not having met these requirements, the consignment may be retained in quarantine, returned to the country of origin or destroyed without recompense.

#### ANIMAL HEALTH CERTIFICATE

Import Permit Number: \_\_\_\_

Species and Category: BOVINE EMBRYOS

Importing Country: AUSTRALIA

Exporting Country: UNITED STATES OF AMERICA

Ministry/Department of: AGRICULTURE Service: APHIS, VETERINARY SERVICES Region/District/Province/State:

#### I. Information concerning each donor

a) Cows:

Breed:

Herd book number:
Identification:
Herd of origin:

b) Bulls:

Breed:

Herd book number: Identification: Herd of origin:

#### II. Information concerning embryos and semen from each donor:

Embryos:

Semen:

Dates of collection: Number of embryos: Number of straws: Straw identification: Date of collection: Number of straws: Straw identification:

III. Origin of the embryos:

Exporter Name:

Address:

Registered name of the approved embryo collection team: Name and address of premises at which embryos collected:

### IV. Destination of the embryos

Consignee Name:

Address: c/o Chief Quarantine Officer (Animals)

[State of Import]

#### V. Sanitary Information

The undersigned embryo collection/production team veterinarian and the undersigned Official Veterinarian certify in respect of the donor animals described in part I(a) of this certificate, and in respect of the bovine embryos/oocytes described in part II of this certificate, that:

1. The United States of America is recognized by the OIE as a foot-and-mouth disease (FMD) free country where vaccination is not practiced, and

Health Certificate #	
(Valid only if USDA Veterinary Seal appears here)	

meets the OIE Code Article definitions for country freedom from:

- rinderpest (Article 2.1.4.2)
- contagious bovine pleuropneumonia (Article 2.1.6.2)
- lumpy skin disease (Article 2.1.7.2)
- Rift Valley fever (Article 2.1.8.2), and
- bovine spongiform encephalopathy (BSE) (Article 3.2.13.2).
- 2. None of the male of female donors has resided in the United Kingdom.
- 3. If from a country with a low incidence of BSE each donor (both male and female):
  - was born after the prohibition in that country, on feeding to ruminants animal feeds containing tissues originating from ruminants;
     has not been resident, since weaning, on a property or premises on which meat meal was fed to ruminants since the respective donor was born;
  - has not originated from, or been part of, a herd in which there has been a confirmed case of BSE;
  - is not the offspring of a BSE suspect or affected dam.
- 4. Each female donor had been continually resident and free from any quarantine restrictions in the United States of America (USA) or Canada for at least 90 days immediately prior to the first collection of embryos.
- 5. Each female donor was inspected by the Team Veterinarian or an Official Veterinarian on each day that embryos were collected for this consignment and was found to be free from signs of infectious or contagious disease.
- 6. Bovine pestivirus (BVD) testing:
  Each female donor gave a negative result to one of the following tests
  for bovine pestivirus using a blood specimen taken between 30 and 14
  days immediately prior to embryo collection: (delete that which is not applicable)
  EITHER:
  - an antigen capture enzyme-linked immunosorbent assay (ELISA) on peripheral blood leucocytes: Date of test:\_\_\_\_\_OR
  - a monoclonal immunoperoxidase (IP) or other virus isolation test on blood or serum: Date of test:\_\_\_\_\_
- 7. Bovine tuberculosis is compulsorily notifiable in the USA.

During the 180 days immediately prior to and during embryo collection, the herds in which each female donor resided were officially free from bovine tuberculosis as defined by OIE Code Article 3.2.3.1., and/or Accredited (tuberculosis free) by the USDA.

8. Vesicular stomatitis was not reported within 80 kilometers of the premises where the male or female donors resided during the period from two months prior to the

Health Certificate #		
(Valid only if USDA Veterinary Seal appears here)		

first collection for this consignment until one month after the final collection for this consignment.

- 9. The semen used to produce the embryos in this consignment was free from quarantine restrictions and was: (delete that which is not applicable) EITHER:
  - collected at a semen collection center which complies with "CSS
     (Certified Semen Services) Minimum Requirements for Disease Control of
     Semen Produced for AI."

OR

- complied with the minimum health standards for semen imported into the USA.
- 10. All the embryos in this consignment were fertilized in vivo, collected, processed, identified, stored and transported in accordance with Code Appendix 4.2.3.1., by an embryo collection team which met the Code conditions and was approved, at the time of collection, by the USDA for the collection of embryos for export. The embryos were treated with trypsin during the washing process as described in Section IV Chapter 2 of the IETS Manual.
- 11. The embryos in this consignment were: (delete that which is not applicable) EITHER
  - not subjected to micromanipulation involving breaching of the zona pellucida and all had intact zona pellucida at the time of storage

OR

Signature:\_\_\_\_

- or met the conditions laid out in Code Appendix 4.2.3.5. for micromanipulated embryos.
- 12. The embryos in this consignment have been stored:
  - only with other embryos or semen collected for export to Australia;
  - in sealed containers:
  - since the end of the collection period until export in an approved secure place.

Date Issued: \_\_\_\_\_

13.	The embryos were identified and placed into new, unused liquid nitrogen in a new or properly disinfected container for export under the supervision of an		
	Official Veterinarian. The container was sealed and the number or mark on the seal is as follows:		
Tean	n Veterinarian:		

	Health Certificate #
Name and address:	
Endorsing Federal Veterinarian	
Signature:	Date endorsed:
Name and address:	

Attachment 2
OIE Code Articles

## Article 2.1.4.2 RINDERPEST

## Rinderpest: free country

A country may be considered free from rinderpest when it has been shown that rinderpest has not been present for at least the past three years.

This period shall be six months after the occurrence of the last case for countries in which a stamping-out policy is practiced, with or without vaccination against rinderpest.

## Article 2.1.6.2 CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP)

## CBPP: free country

A country may be considered free from CBPP when it has been shown that CBPP is not present and that one year has elapsed after the occurrence of the last case for countries in which a stamping-out policy is practiced.

# Article 2.1.7.2. LUMPY SKIN DISEASE (LSD)

## LSD: free country

A country may be considered free from LSD when:

- 1) LSD is notifiable in the country;
- 2) no case of LSD has been confirmed for at least the past three years.

## Article 2.1.8.2 RIFT VALLEY FEVER (RVF)

## RVF: free country

A country may be considered free from RVF when RVF is compulsorily notifiable, when no case, either clinical or serological, has been confirmed for the past three years and when the country has not imported any susceptible animals from a country considered infected with RVF during this period.

# Article 3.2.13.2. BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

## BSE: free country

Countries may be considered free of BSE if:

1) there has been no clinical case of BSE, the disease is notifiable, and an effective and continuous surveillance and monitoring system is practiced; or

all cases of BSE have been clearly demonstrated to originate directly from the importation of live cattle from countries where BSE has been reported, provided that the disease is made notifiable and suspect animals are slaughtered, investigated and, if disease is confirmed, completely destroyed and an effective and continuous surveillance and monitoring system is practiced.

## Article 4.2.3.1 THE COLLECTION AND PROCESSING OF BOVINE EMBRYOS/OVA

#### A. AIMS OF CONTROL

The purpose of official sanitary control of embryos/ova intended for movement internationally, is to ensure that specific pathogenic organisms which could be associated with embryos/ova are controlled, and the risk of infection being transmitted to recipient animals and progeny is reduced to an acceptable level.

The disease situation between exporting and importing countries may be similar or dissimilar, and national prophylactic programs can vary widely, as can vaccination and testing requirements. Thus, exporting and importing countries may have different conditions for the approval of embryo collection teams and associated processing laboratories. For these and other reasons, the Appendix covers the main sanitary conditions under which embryos/ova may be collected, processed and transported.

#### B. GENERAL CONDITIONS

The Veterinary Administration should ensure that the general conditions relating to animal health set out in the following paragraphs are followed for the international movement of embryos/ova.

- 1. Embryo collection team
  Embryo collection team means a group of competent technicians including at least one veterinarian to perform the collection, processing and storage of embryos. The following conditions should apply:
- a) This team should be supervised by a team veterinarian.
- b) The team veterinarian is responsible for all team operations which include sanitary handling and surgery of donors and disinfection and hygienic procedures.
- c) The team veterinarian should be specifically approved for this purpose by an Official Veterinarian.
- d) Team personnel should be adequately trained in the techniques and principles of disease control. High standards of hygiene should be practiced to preclude the introduction of infection.
- e) The collection team must have adequate facilities and equipment for:
  - collecting embryos;
  - processing and treatment of embryos at a permanent site or mobile laboratory;
  - sorting embryos.
- f) As these facilities are not necessarily at the same location, the collection team must keep a record of its activities which must be maintained for inspection by the approving authority

- for a period of two years after the embryos have been exported.
- g) The collection team should be subjected to regular inspection to ensure compliance with sanitary collection, processing and storage of embryos.
- h) The collection team must not operate in an infected zone unless the disease in question has been listed by the International Embryo Transfer Society (IETS) in category one\*.

# 2. Processing laboratories

The processing laboratory may be mobile or permanent. It is a premises in which embryos/ova are recovered from collection media, examined, washed, and subjected to any required treatments before freezing, storage, and quarantine pending results of health control tests.

A permanent laboratory may be part of a specifically designed collection and processing unit, or a suitably adapted part of an existing building. It may be on the premises where the herd of donor animals is kept. In either case, the laboratory should be physically separated from animals. Both mobile and permanent laboratories should have a clear separation between dirty areas (animal handling) and the clean processing area.

- a) The laboratory should be under the direct supervision of the team veterinarian and regularly inspected by an Official Veterinarian.
- b) While embryos/ova for export are being handled prior to storage in ampoules/straws, no embryos/ova of lesser health status should be processed.
- c) The laboratory should be protected against rodents and insects.
- d) The processing laboratory should be constructed with materials which permit its effective cleansing and disinfection. This should be done following each occasion on which embryos are processed.
- e) The laboratory must not be situated in an infected zone (foot-and-mouth disease, rinderpest).
- 3. Donor animals
- a) At the time of collection, donor animals should be clinically inspected by the team veterinarian and confirmed to be free of contagious and infectious diseases transmissible to cattle.
- b) The herd of origin must be free of clinical signs of foot-and-mouth disease, rinderpest, and contagious bovine pleuropneumonia and must not be situated in an infected zone (foot-and-mouth disease, rinderpest) for the 30 days before and after collection, unless the disease in question has been listed by the IETS in category one.
- c) The Veterinary Administration should have knowledge of, and authority over, the herd of origin of the donor animals.
- d) The donor animals should not have been imported from another country during the previous 60 days and should have been in the herd of origin for at least 30 days.
- e) The owner of donor animals should sign a statement that to the best of his knowledge the donors are free of any known genetic defects and not associated with such defects in close relatives.

## 4. Testing of donor animals and embryos/ova

There are two main approaches to ensuring embryos/ova are free of pathogenic organisms. The traditional method is based on the testing of donor animals over extended periods of time and is

applied only to diseases not listed in category one and determined on the basis of their pathogenesis to pose more than a negligible risk of transmission by embryos. The checking of paired sera and the reapplication of other tests may be required after normal incubation periods to determine the health status of donors. The other method is based on recent well-documented work which identifies the high measure of freedom from specified bacteria and viruses of embryos/ova which have been processed in accordance with the IETS Manual. It obviates long periods of isolation and repeated testing of donor animals and some of the inequities of testing of serum samples to determine disease status, provided they satisfy the basic criteria set out in paragraph 3 of this Appendix.

- a) Traditional method
  - i) Testing of live donors by bilateral agreement:

The holding of frozen embryos/ova in flasks of liquid nitrogen for periods which cover the normal incubation period of those diseases of concern to an importing country provides the opportunity to test sera at/or prior to and after collection from donor animals.

ii) Semen used to inseminate donor animals artificially or fertilize ova should meet the health requirements and standards set out in Appendix 4.2.1.1. and 4.2.1.2.

When frozen semen used to inseminate donor animals was collected from bulls no longer living and when the health status of the bulls concerning a particular infectious disease or diseases was not known at the time of collection, additional tests may be required of the inseminated donor female after ova/embryo collection to verify that these infectious diseases were not transmitted. An alternative may be to subject the semen to testing. Where natural service or fresh semen is used, sires should meet the same health requirements as donor females.

b) New method: processing of embryos/ova

The zona pellucida of each embryo/ovum must be examined over its entire surface area at not less than 50X magnification and certified to be intact and free of adherent material. The embryos/ova must be washed according to the IETS Manual and have intact zona pellucida before and after washing. Only embryos/ova from the same donor should be washed together. All shipments of embryos/ova must be accompanied by a statement signed by the team veterinarian in charge of the processing certifying that these examinations have been completed.

# 5. Collection and storage of embryos/ova

a)Media

Any biological product of animals origin used in the collection, processing, washing or storage should be free of living micro-organisms. Media and solutions used in the collection, freezing, and storage of embryos/ova should be sterilized by approved methods according to the IETS Manual and handled in such a manner as to ensure that sterility is maintained. Antibiotics should be added to collection, wash and storage media according to the IETS Manual.

# b) Equipment

All equipment used to collect, handle, wash, freeze, and store embryos/ova should be sterilized prior to use according to the IETS Manual.

- 6. Optional tests and treatment
- a) The examination of the embryos/ova, collection of washing fluids can be requested by an importing country. Tests may be carried out on these fluids to confirm that the degree of

quality control of the collection team is at an acceptable risk level:

i) Embryo/ova

Where embryos are being collected exclusively for export, all non-fertilized ova and degenerating embryos collected from a donor should be washed according to the IETS Manual and pooled for possible testing.

ii) Collection fluids

The collection fluid should be placed in a sterile container, such as a measuring cylinder, and allowed to stand undisturbed for one hour. The supernatant fluid should then be removed and the bottom 100 ml, along with accumulated debris, decanted into a sterile bottle. If a filter is used in the collection of embryos/ova, then any debris that is retained on the filter must be rinsed into the 100 ml of retained fluid.

iii) Washing fluids

The last four washed of the embryos/ova (washes 7, 8, 9, and 10) should be pooled (IETS Manual).

iv) Samples

The samples referred to above should be stored at 4°C and tested within 24 hours. If this is not possible, then samples should be stored frozen at least -70 degrees Celsius.

- b) Treatment of the embryos/ova with the enzyme trypsin may be required. This treatment should be carried out according to the IETS Manual.
- c) Only embryos/ova from one donor should be processed simultaneously.
- 7. Storage, quarantine and transport
- a) The embryos/ova should be stored in sterile ampoules/straws in sterilized liquid nitrogen containers under strict hygienic conditions at a storage place, approved by the Veterinary Administration of the exporting country, where no risk of contamination of the embryos/ova can occur.
- b) Only embryos/ova from the same donor should be stored together in the same ampoule/straw.
- c) Ampoules/straws must be sealed at the time of freezing and should be labelled according to the IETS Manual. The liquid nitrogen container should be sealed prior to shipment. d)Embryos/ova should be frozen in fresh alcohol or liquid nitrogen and stored in fresh liquid nitrogen in sterilized flasks.
- e) Stored embryos/ova must not be exported until health certification is completed.
- \* Based on available research and field information the IETS has placed the following disease/disease agents of cattle in category one: enzootic bovine leucosis, foot-and-mouth disease, bluetongue, Brucella abortus, infectious bovine rhinotracheitis (trypsin treatment required). This indicates that sufficient evidence has occurred to show that the risk of transmission is negligible provided that the embryos are properly handled\*\* between collection and transfer.
- \*\* Manual of the International Embryo Transfer Society, 1990.

Appendix 4.2.3.5 COLLECTION AND PROCESSING OF MICROMANIPULATED BOVINE EMBRYOS

Appendix 4.2.3.1. covers the official sanitary control measures for the international movement of intact bovine embryos, but it does not apply to embryos/ova which have been subjected to sexing, splitting (twinning), cloning (nuclear transplantation) or other manipulations which interfere with the integrity of the zona pellucida. Such embryos are subsequently referred to here as "micromanipulated embryos/ova".

To bring micromanipulated embryos/ova within the scope of the above mentioned Appendix the following conditions shall apply.

#### A. GENERAL CONDITIONS

The Veterinary Administration should ensure that the general conditions for animal health set out in the following paragraphs are adhered to for the international movement of micromanipulated embryos:

- a) Prior to any micromanipulation which involves breaching of the zona pellucida, all embryos/ova must be collected and processed according to the sanitary conditions laid down in Appendix 4.2.3.1.
- b) Responsibility for the embryos/ova must remain with the embryo collection team, and all processes involving micromanipulation should be carried out in an approved processing laboratory under supervision of an approved team veterinarian (see paragraphs B1 and B2 of Appendix 4.2.3.1.).
- c) Donor animals must comply with the conditions laid down in paragraph B3 of Appendix 4.2.3.1. and the criteria for testing and/or other methods to ensure that embryos/ova are free of pathogenic organisms are as described in paragraph B4 of that Appendix.
- d) All embryos to be micromanipulated must be washed according to the protocols laid down in Chapter II of the IETS Manual (1990) and they must be observed to have intact zonae pellucidae before and after washing. Only embryos/ova from the same donor female should be washed together at the same time. After washing but before micromanipulation the zona pellucida of each embryo/ovum should be examined over its entire surface area at not less than 50X magnification and certified to be intact and free of adherent material.
- e) If surrogate zonae are used, they should be of bovine origin and derived and treated in the same manner as other zonae prior to micromanipulation.

#### B. PROCEDURES FOR MICROMANIPULATION

## a) Media

Any biological product of animal origin used in the collection, washing, treatment, micromanipulation, culture, storage or transport of the embryos/ova should be free of living micro-organisms. All media and solutions which are used should be sterilized by approved methods according to the IETS Manual, and should be handled in such a manner as to ensure that sterility is maintained. Antibiotics should be added to the media as recommended in the IETS Manual.

## b) Equipment

Separate equipment should be used to micromanipulate embryos/ova from different donor animals for whatever purpose and must be sterilized prior to use in accordance with recommendations in

the IETS Manual.

## c) Nuclei for transfer

Where it is intended to transplant nuclei derived either from the blastomeres of morula-stage embryos or from stem cells derived from pre-hatching stage (i.e. zona pellucida intact) embryos, the parent embryos from which those nuclei are derived should fulfil the conditions of this document. Similarly, where it is intended to transplant a nucleus beneath the zona pellucida for fusion with an enucleated oocyte, that oocyte should be collected, cultured, and manipulated and the resulting embryo culture according to recommendations presented in this Appendix, or in Appendix 4.2.3.4.

## C. OPTIONAL TESTS AND TREATMENTS

The importing country may request that tests\* be carried out on certain samples, or that embryos/ova are treated to ensure that specified pathogenic organisms are absent.

a) Samples

Samples to be treated may include those referred to in paragraph B6 of Appendix 4.2.3.1. Samples collected after micromanipulations are unlikely to require testing if the foregoing conditions are met.

b) Treatments

Treatments of embryos/ova with substances such as the enzyme trypsin or other substances proven to inactivate or remove pathogenic organisms and that are harmless to the embryo may be requested, but these also should be applied prior to any micromanipulation, and according to the IETS Manual.

## D. STORAGE, QUARANTINE AND TRANSPORT

Micromanipulated embryos/ova should be stored, quarantined and transported according to the conditions laid down in paragraph 7 of Appendix 4.2.3.1. Health certification documents should contain full details of all micromanipulations, and where and when they were carried out.

\* If the samples mentioned above in paragraph 3 are to be tested for pathogenic agents, then the microbiological techniques in current use for those agents would be appropriate.